The Relevance of Prandial State for Peripheral Insulin-Dependent Glucose Uptake
A Relevância do Estado Prandial para o Aporte Periférico de Glucose por via Insulinodependente

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RESUMO

A acção da insulina é um processo essencial para o metabolismo glicídico e para a homeostase da glucose em particular.

Recentemente, tem sido reconhecido um papel preponderante do estado nutricional para a acção da insulina, sendo dada uma relevância cada vez maior aos estudos realizados no estado pós-prandial, em detrimento do estado de jejum. De facto, se por um lado o pâncreas é essencial para a secreção de insulina, por outro lado, o fígado parece ser importante na acção periférica (extra-hepática) da insulina, através de uma via que apenas está activa após a refeição e que, quando alterada, induz ou agrava a condição de insulinorresistência. Tal é compatível com as recentes observações de que as anomalias metabólicas que ocorrem numa fase de pré-diabetes são primeiramente evidentes no estado pós-prandial.

Na presente revisão irão abordar-se os principais mecanismos de acção hipoglicemiante da insulina numa perspectiva do organismo (*whole-body*), dando particular ênfase às diferenças entre a acção da insulina no estado de jejum e no pós-prandial. Em particular, discutir-se-á como diferentes tipos de refeição afectam a sensibilidade à insulina pós-prandial e qual a relevância do mecanismo dependente da substância hepática sensibilizadora da insulina (HISS) na acção da insulina.

As abordagens no tratamento da insulinorresistência têm-se centrado quase exclusivamente na insulina, tendo por base estudos realizados numa situação de estado estacionário (jejum). Espera-se que a presente revisão permita servir de ponto de partida para novas abordagens e terapêuticas para reversão da condição de insulinorresistência.

PALAVRAS-CHAVE

Insulina; Acção da insulina; Via de transdução de sinal da insulina; Estado prandial; Substância hepática sensibilizadora da insulina (HISS).

ABSTRACT

Insulin action is an essential process for carbohydrates metabolism and for glucose homeostasis in particular. Recently, it has been recognized the vital importance of the nutritional state for insulin action, being given a higher relevance to the postprandial state in comparison with the fasted state.

In fact, if the pancreas is essential for insulin secretion on one hand, on the other it has been also described an hepatic pathway that contributes to increase peripheral (extra-hepatic) insulin action following a meal. Impairment of this hepatic pathway leads to or aggravates insulin resistance, which is in accordance with those reports claiming that the metabolic anomalies observed in the course towards diabetes are first seen in the postprandial state.

In this review it will be provided a brief overview of the major mechanisms of insulin hypoglycemic action in a whole-body perspective, giving particular emphasis to the differences in insulin action between fasted and postprandial states. In particular, it will be discussed how different meal compositions affect postprandial insulin sensitivity and what is the relevance of the hepatic insulin sensitizing substance (HISS)-dependent mechanism for insulin action.

Until now, approaches to the treatment of insulin resistance have been focused almost exclusively on insulin, being based on studies performed in a steady-state (fasted state). It is expected that the present review may serve as a starting point for new approaches and therapeutics for reversion of insulin resistance conditions.

KEYWORDS

Insulin; Insulin action; Prandial state; Hepatic insulin sensitizing substance (HISS).

INTRODUCTION

Insulin action was discussed in the previous review from a molecular and cellular perspective, supported in information obtained mainly from *in vitro* studies. However, insulin action, in particular its hypoglycemic action, can be studied at the level of tissues, organs and whole-body (*ex vivo* and *in vivo* studies). Insulin role in glucose uptake has been studied in all sorts of systems, such as tissues[1] or isolated organs maintained under perfusion[2].

It is unquestionable that the most objective way of performing these studies is *in vivo*, despite of a higher degree of complexity. Several methodologies are described to assess insulin sensitivity *in vivo*, both in animals and humans. For a review of the methodologies available to assess insulin sensitivity, consult additional publications[3-6].

Insulin hypoglycemic action involves glucose distribution from the blood circulation to tissues/cells. It is reasonable to assume that this insulin-dependent glucose uptake by the tissues is more relevant when the glycemia rises, which, under physiological conditions, occurs after a meal (*e.g.*). Thus, the essential role played by insulin in glucose homeostasis is particularly important in the postprandial state, not only because it has an inhibitory action upon hepatic glucose production and output, but mainly because it stimulates glucose uptake by peripheral tissues, namely skeletal muscle and adipose tissue. Indeed, it is now assumed that reducing post-meal glucose excursions is rather difficult, although extremely important in people with diabetes and impaired glucose tolerance[7-10].

IMPORTANCE OF THE PRANDIAL STATE FOR HYPOGLYCEMIC ACTION OF INSULIN

Until recently, most diagnostics were dependent essentially on fasting plasma levels of glucose and/or insulin. However, a better understanding of the pathophysiology behind diabetes lead us to the observation that type 2 diabetes is characterized by a decline in insulin secretion, essentially in response to nutrient ingestion[11]. In fact, the major complications related with insulin resistance are primarily observed in the

postprandial state[9,12-15], which reflects a higher need for insulin action. Thus, the traditional focus on the fasted state is inconsistent with recent data, which indicates that the metabolic defect in the pre-diabetic condition relates more strongly to postprandial deficiency than to the fasting state[12,16].

It is estimated that 54 to 67% of the people with reduced glucose tolerance present a normal fasting glycemia[16]. Furthermore, a meta-analysis of 20 European studies revealed that about 31 % of the people that were diagnosed with diabetes using the postprandial glycemia had also normal fasting glycemia[16], suggesting a clear difference in the regulation of glucose homeostasis before and after a meal. On the other hand, fasting insulinemia, which has also been used as a surrogate for insulin resistance, bears a nonlinear relationship to insulin action directly measured[17] and its applicability also seems to fail when there is even subtle β-cell failure[18].

The awareness of the importance of postprandial insulin action and glucose homeostasis has led to recent guidelines issued by the International Diabetes Federation (IDF), aiming to control postprandial blood glucose levels[7].

In the past decade, several studies have highlighted the importance of postprandial insulin action. It has been observed that under physiological conditions insulin action is significantly higher in the postprandial than in the fasted state[11,19-21]. Indeed, although there is no consensus about which nutrients or types of meal are the most effective in producing the increment in insulin action, the majority of the studies that compare insulin sensitivity before and after a meal suggest that insulin action is higher in the postprandial state[11]. Animal experiments have demonstrated that insulin action increases following a meal, when this meal was provided *ad libitum, i.e.*, without restriction[19,20,22], when it was delivered endogastrically by gavage[19] or when it was given intragastrically via a surgically-placed gastric catheter[21]. Additionally, experiments performed by our group suggested that for the meal-induced insulin sensitization to occur, the meal must reach the intestine (Afonso and Macedo, unpublished observations).

Recently, this same meal-effect on insulin sensitivity was also shown in humans[23]. In healthy individuals, with a body mass index (BMI) of 23.3±0.8 kg/m^2, insulin sensitivity was assessed before (24 h fasting) and after ingestion of a standard test meal; it was observed that insulin sensitivity in the postprandial state was about 3 times higher than that in the fasted state[23]. When the same protocol was applied to individuals with excess weight (BMI of 27.7±0.4 kg/m^2), a condition usually associated with the decrease of insulin sensitivity, insulin action in the fasted state was similar to that observed in normal-weight individuals; however, the excess-weight individuals presented a significant impairment of the postprandial insulin sensitivity (Patarrão and Macedo, unpublished observations).

These data, both animal and human, suggest that early stages of insulin resistance may already exist, but can only be detected in the postprandial state. Thus, from a clinical point of view, it becomes particularly relevant to assess insulin action in the postprandial state, since it is when the first manifestations of insulin resistance seem to occur, still in a pre-diabetic and asymptomatic stage.

THE RELEVANCE OF MEAL COMPOSITION FOR POSTPRANDIAL INSULIN ACTION

Mixed-meals, composed by proteins, lipids and carbohydrates, were used in the experiments described in the previous section. Further evaluation of the impact of meal composition on insulin action revealed that meals composed by carbohy-

drates only (glucose or sucrose) do not induce an increase in insulin action from fasted to fed state[21].

Indeed, unlike mixed-meals, glucose and sucrose are clearly incapable of producing the prandial signal that leads to potentiation of insulin action after a meal[21]. Therefore, neither glucose nor sucrose seems to be adequate to mimic the postprandial condition.

This is very important from a clinical point of view, since it suggests that studies of insulin action using glucose test meals, such as the oral glucose tolerance test (OGTT), are not the most appropriate to evaluate insulin action in the postprandial state, unlike tolerance tests that that use mixed-meals, *i.e.*, meal-tolerance tests (MTT). According to other authors, the mixed-meal-based tests (MTT) provide higher insulin sensitivity indexes than the OGTT or the hyperglycemic clamp[24]. Probably due to this, the use of mixed-meals allow greater capacity to simultaneously detect differences in both glycemic and insulinemic profiles than OGTT, in particular in insulin resistant models[25].

ROLE OF THE AUTONOMIC NERVOUS SYSTEM IN GLUCOSE HOMEOSTASIS

Autonomic nervous system is the major mediator of physiological responses to both internal and external stimuli.

Autonomic nervous system can be divided in sympathetic (SNS) and parasympathetic nervous system (PNS), both of which enervate the GI tract. PNS and SNS usually act in a reciprocal way, *i.e.*, the increase in activity of one of them is accompanied or induces a decreased activity of the other[26]. However, although the reciprocity between sympathetic and parasympathetic nervous systems is the general rule, there are some exceptions, in certain physiological conditions; *e.g.*, in hypoglycemic conditions, activation of both sympathetic and parasympathetics induces the release of glucagon by α-pancreatic cells[26].

Generally speaking, regulation of glycemia and glucose homeostasis, one can consider that SNS and PNS have antagonic effects: usually, the PNS acts towards facilitation of peripheral glucose uptake, whereas the SNS leads to a rise in glycemia, through reduction of glucose uptake and increase of hepatic glucose production.

SNS contribution for regulation of glycemia and peripheral glucose distribution is done, for instance, by control of the vascular tone, which regulates blood flow to a particular system or tissue, but mostly through its hepatic effects. Indeed, under stress and/or hypoglycemic conditions, SNS activation leads to stimulation of both glycogenolysis and gluconeogenesis, therefore increasing glucose efflux from the liver into the circulation. Gardemann and Jungermann (1986) determined that the neural-induced increase in glucose output from the liver is due to SNS rather than PNS activity, since they observed that hepatic neuronal stimulation, after α- and β-adrenergic blockade, did not induce hepatic glucose output[27]. Nevertheless, others claim that stimulation of hepatic glycogenolysis and gluconeogenesis by SNS activation have a small contribution to the hepatic response to hypoglycemia, as long as the hormonal regulatory systems remain intact[28]. Thus, regulation of the endogenous glucose production seems to be mediated essentially by modulation of insulin (decrease) and glucagon (increase) secretion, and by the SNS[27].

Activation of the PNS (vagal) occurs during ingestion of a meal[26,29]. The vagus nerve innervates almost all organs involved in digestion, absorption and metabolism of nutrients. Therefore, it profoundly influences the metabolic processing of food[26]. Several authors have observed that the PNS plays a key role in regulation of glycemia through pancreas and liver innervations[16,26,27,30,31]. Indeed, vagal electrical stimu-

lation seems to increase glucose or meal-induced insulin secretion[32], which is impaired by muscarinic antagonism[33], although these observations are not consensual[34,35]. Interestingly, prolonged hyperinsulinemia can lead to impairment of parasympathetic signaling[36], which can explain eventual autonomic nervous dysfunctions in pathophysiological conditions associated with hyperinsulinemia, such as obesity[37]. In addition, specifically at the hepatic level, Gardemann and Jungermann (1986) observed that parasympathetic stimulation has direct effects on glucose metabolism[27], which are synergistic with insulin action and antagonic from glucagon action, such as activation of glycogenesis and inhibition of glycogenolysis and gluconeogenesis[26,38]. Xie and co-workers further observed that surgical ablation of hepatic parasympathetic nerves reduces peripheral insulin sensitivity in the postprandial state[30], which was reversed by intraportal acetylcholine administration[39].

RELEVANCE OF THE LIVER FOR INSULIN ACTION AND GLUCOSE HOMEOSTASIS

The central role of the liver in glucose uptake by peripheral (extra-hepatic) tissues has long been acknowledged by several authors[2,27,28,31,40-42].

In the fifties, Lang and co-workers observed that performing an hepatectomy in healthy animals leads to an impairment of the peripheral glucose uptake of about 60%, which could not be reversed by high-dose insulin administration[40]. These authors suggested at that time that the liver releases a humoral agent capable of potentiating glucose uptake by extra-hepatic tissues[40]. In the following decade, Mertz e Schwartz confirmed the essential role of the liver in peripheral glucose uptake, through the observation that the reduction of glucose uptake in eviscerated was reversed by administration of an extract of fresh liver[2,41].

In the last decade of the past century, Petersen e Tygstrup observed that the inclusion of isolated rat liver in a skeletal muscle preparation with a recirculatory system significantly increased glucose uptake by the skeletal muscle[2]; these authors further observed that the rate of glucose incorporation into the muscle decreases when the liver was removed from the system, presenting a 30-min half-life, suggesting that the liver releases a humoral factor which increases glucose uptake by the skeletal muscle[2].

The hypothesis that the process of insulin-dependent glucose uptake depends not only on insulin action *per se*, but also on an hepatic mechanism that potentiates insulin hypoglycemic action, has evolved enormously mainly through the contribution of Lautt and co-workers in the past 15 years. In 1993, these investigators realized for the first time that hepatic parasympathetic nerves are essential for peripheral glucose uptake and homeostasis[30], which was also confirmed by Moore and co-workers[31]. This parasympathetic activity is part of a pathway that is specifically stimulated in the postprandial state and it is responsible for 50 to 60% of insulin hypoglycemic action after a meal[19]. Lautt and co-workers then suggest that such pathway involves the release of an hepatic humoral factor, which potentiates peripheral insulin action[19,43,44].

Thus, meal-induced increment in insulin sensitivity seems to rely mostly on an hepatic mechanism that involves hepatic parasympathetic nerves and culminates in the release of a humoral factor – the hepatic insulin sensitizing substance (HISS) –, which potentiates peripheral insulin action.

One can consider that insulin hypoglycemic action in the postprandial state can be divided in two components, which act synergistically to increase glucose uptake. The first component is insulin

action *per se*, which corresponds to direct action of the insulin molecule on target cells, stimulating its receptor and activating the signaling transduction pathway(s). The second component is a HISS-dependent component of insulin action, in which HISS release from the liver is required to potentiate peripheral insulin hypoglycemic action.

THE HISS PATHWAY AND MEAL-INDUCED INSULIN SENSITIZATION

Meal ingestion promotes insulin secretion from the pancreas and activation of the PNS[26]. This parasympathetic activation is extremely important for glucose homeostasis[26,30,43], since it triggers the mechanism of HISS synthesis, action of which is essential for postprandial insulin sensitivity[45]. Indeed, Lautt's group observed that surgical ablation of hepatic anterior plexus induces pronounced peripheral insulin resistance, which is caused specifically by the loss of parasympathetic function[30,46].

This hepatic parasympathetic reflex seems to be mediated by muscarinic receptors present in the liver, reason why intraportal atropine administration induces significant peripheral insulin resistance[47]. Atropine-induced insulin resistance is dose-dependent[47,48] and does not aggravate insulin resistance caused by ablation of hepatic anterior plexus[43,47]. Furthermore, intraportal, but not intravenous, acetylcholine administration completely reverses insulin resistance induced either by ablation of hepatic anterior plexus or by atropine administration[39]; on the other hand, acetylcholine administration to animals with intact hepatic parasympathetic nerves does not have any additional effect on insulin sensitivity[39].

Many of acetylcholine biologic actions are mediated by nitric oxide (NO)[49]. Indeed both L-NMMA and L-NAME, competitive inhibitors of NO synthase (NOS), reduce insulin sensitivity, but only when administered intraportally[50]. Furthermore, hepatic NOS inhibition in animals previously submitted to hepatic parasympathetic denervation does not produce any additional insulin resistance, whereas intraportal administration of the NO donor SIN-1 completely reversed insulin resistance caused either by hepatic NOS inhibition or hepatic denervation[50]. Additional experiments performed by our group showed that hepatic acetylcholine induces hepatic NOS activation and consequently increases NO synthesis, and not the other way around[51], as described by others for NO metabolism in different systems[52,53].

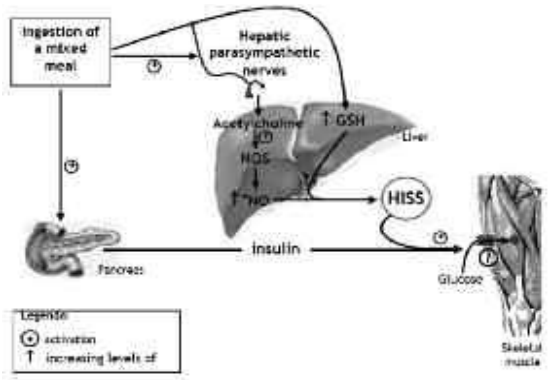
Thus, parasympathetic nerves induce the release of acetylcholine in the liver, leading to an increase in hepatic NO synthesis, which seems to be necessary for HISS synthesis.

Additionally, several authors observed that depletion of hepatic glutathione levels (GSH, reduced form) directly leads to impairment of glucose tolerance[54]. In fact, our studies showed that hepatic GSH depletion through inhibition of its synthesis leads to a condition of insulin resistance similar to that produced by hepatic NOS inhibition, which is not reversed simply by SIN-1 administration[55], suggesting that both NO and GSH are required, but not sequential steps in HISS synthesis. This was later confirmed by the observation that intraportal co-administration of NO and GSH donors increases insulin sensitivity, which does not occur when those donors are given separately or intravenously[56]. Recent experiments performed by Punithavathi and co-workers support these conclusions, since they observed that reversion of insulin resistance in several animal models implies the increase of GSH levels[57].

In summary, from what is known so far, HISS synthesis is initiated following meal ingestion, through an hepatic parasympathetic reflex that involves acetylcholine release in the liver. Acetylcholine activates hepatic muscarinic receptors, stimulating hepatic NOS and NO production, which, in

the presence of GSH, leads to HISS synthesis. HISS is then released from the liver potentiating peripheral insulin action, namely at the skeletal muscle. The known steps in HISS synthesis and action are represented in figure 1.

FIGURE 1



Pathway of hepatic insulin-sensitizing substance (HISS). In the postprandial state, hepatic parasympathetic nerves are stimulated and the resultant acetylcholine binds to muscarinic receptors, activating hepatic nitric oxide synthase (NOS) and consequently increasing nitric oxide (NO) production. In addition to NO, hepatic glutathione (GSH) is also essential for the synthesis and/or release of HISS from the liver. HISS induces an increase in postprandial insulin-dependent glucose uptake in 50-60%, by acting primarily in the skeletal muscle (adapted from[81]).

HISS action seems to be directed mainly to skeletal muscle, the major tissue responsible for glucose uptake[43], as represented in figure 1. The work performed by Petersen e Tygstrup in 1994 already suggested that a humoral factor released by the liver increases glucose uptake in skeletal muscle[2]. Afterwards, using arterial-venous gradients, Xie and Lautt demonstrated that the main site affected by HISS inhibition is the skeletal muscle, suggesting that this organ is the main target for HISS action[43]. This is consistent with the observations that the skeletal muscle is the major glucose uptaker by an insulin-dependent mechanism[58-60]. Alterations of glucose uptake by the muscle are also the primer responsible for postprandial hyperglycemia in insulin resistante states[58,61].

HISS-dependent insulin action is regulated by the prandial state, being maximal after a meal and inhibited/absent by fasting[19,20,62]. Indeed, HISS action increases significantly 1-2 h after food ingestion, both when the access to nutrients is *ad libitum* and when the food is provided by gavage (endogastric administration)[19]. In rats tested after different fasting periods (0 h, 6 h, 18 h and 24 h), it was observed that insulin sensitivity decreases progressively with the duration of fasting, which was caused by a progressive decline in HISS action[19]. HISS-independent insulin action, on the other hand, is not affected by the prandial state[19].

Additionally, according to our studies, meal composition is also an important regulating factor on HISS pathway activation (figure 1). In fact, carbohydrate meals are not effective triggers of HISS mechanism, whereas mixed meals are capable of stimulating HISS synthesis and therefore allowing for meal-induced insulin sensitization to occur[21]. Indeed, neither intragastric glucose nor sucrose induced a normal increment of fasted insulin action, but a meal composed of proteins, lipids and carbohydrates, administered intragastrically did increase peripheral insulin sensitivity significantly, in a volume-independent manner[21]. Furthermore, denervation of hepatic anterior plexus (parasympathetics) prevents meal-induced insulin sensitization and either hepatic parasympathetic denervation or atropine administration, performed after mixed-meal ingestion, inhibited insulin sensitivity to levels similar to those observed in the fasted state, suggesting the involvement of HISS mechanism[21].

As previously noted, these observations are extremely relevant from the nutritional and clinical perspective, because one of the most widely used tolerance tests, the OGTT, does not take in consideration either the fact that insulin action is higher in the fed than in the fasted state, or the fact that HISS is required for maximal insulin action, which is not triggered by carbohydrates alone. Indeed, if glucose is not an efficient activator of HISS pathway, OGTT neglects the HISS-dependent component of insulin action, which represents 50-60% of total

insulin action in the postprandial state. Thus, eventual alterations in HISS synthesis and/or action cannot be detected by the OGTT, not allowing an early identification of a pre-diabetes condition, which would have been detected through the use of a mixed-meal.

Subsequent studies revealed that the meal must reach the intestine to properly trigger HISS synthesis/action and stimulating meal-induced insulin sensitization (Afonso and Macedo, unpublished observations). This is consistent with the concept that, although the stomach is the main organ in digestion, it is in the small intestine where most nutrient absorption occurs and different intestinal portions are responsible for the absorption of different nutrients and for secretion of different substances, which vary according with prandial state[63,64]. On the other hand, it is described that PNS, which is essential for HISS pathway, is modulated by the prandial state[26]. The presence of certain aminoacids in portal circulation seems to activate hepatic parasympathetics[65] and different nutrients or hormones in portal circulation, such as glucose, aminoacids and somatostatin, affect hepatic parasympathetics[66]. Additional studies also associate hepatic GSH levels with the prandial state[55,67].

Thus, HISS is inactive in the fasted state and it is released from the liver after a mixed-meal. This seems to be an important physiological mechanism to selectively increase glucose uptake by the skeletal muscle when glucose absorption is high, *i.e.*, after a meal, and to diminish glucose uptake in sparing conditions, such as the fasted state (figure 1).

The lack or impairment of HISS-dependent insulin action was shown to contribute for postprandial insulin resistance in several pathophysiological conditions usually associated with insulin resistance and/or type 2 diabetes. Indeed, HISS pathway decreases with aging[20] (Lautt *et al*, 2008), high-sucrose feeding[68], hypertension[69,70] and obesity[37,71,72].

In obesity in particular, HISS impairment seems to be the main contributor for the observed postprandial insulin resistance. In fact, HISS action is significantly impaired both in genetic obesity[71] and high-fat diet-induced obesity[37]. Moreover, our studies have demonstrated that the HISS-dependent insulin action is negatively correlated with both whole-body and abdominal adiposity, suggesting a clear relationship between fat mass accumulation and HISS pathway impairment (Afonso *et al*, unpublished observations). In humans, we have also observed that excess weight is associated with impaired insulin sensitivity, for which the decrease in HISS action is an important factor (Patarrão and Macedo, unpublished observations).

HISS-dependent component is already described in several animal models, such as cats[30,47], rats[19,43,44], dogs[31] and mice[73], with no differences observed between males and females[20,74]. Recently, experiments performed by our group revealed the existence of the HISS pathway also in humans[23,75].

HISS AND POSTPRANDIAL POTENTIATION OF INSULIN ACTION: GUT-BRAIN-LIVER AXIS?

Gastrointestinal tract, enteric nervous system and central nervous system(1) seem to be involved in a two-way communication (efferent and afferent)[76], composed by sympathetic, parasympathetic and afferent sensorial fibers, required for the so-called gut-brain axis signaling[77]. Gut (enteric) hormones also seem to stimulate sensorial nerves and activate autonomic reflexes in the postprandial state[78]. Although it is not

(1) The term "enteric nervous system" was proposed by Wingate in 1981, to characterize a third division of the autonomic nervous system, formed by a neuronal network, intrinsic to the gastrointestinal tract[75].

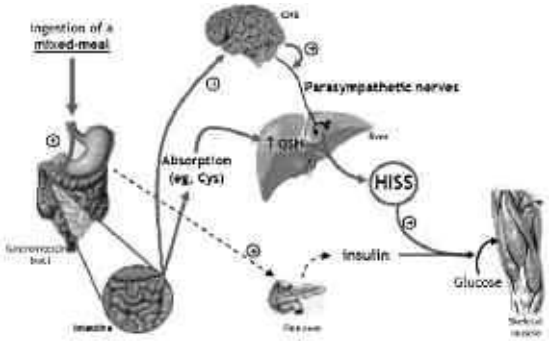
yet clear how this mechanism contributes for glucose homeostasis, it seems to require an interaction between gastrointestinal tract, central nervous system and effector organs involved in regulation of glycemia, namely pancreas and liver. Some authors suggest that this autonomically-mediated metabolic reflex is anticipatory, since it allows the peripheral tissues to prepare for the adequate handling of nutrients[79]. They also claim that diabetes is associated with impairment of such anticipatory reflex[79].

The gut-brain-liver interactions seem to be mediated by autonomic nervous system, and by the PNS in particular, which is stimulated by ingestion of a meal and it is involved in peripheral glucose uptake[21]. As previously mentioned, the prandial signal that leads to HISS synthesis depends on elevation of hepatic GSH and hepatic parasympathetic activation, both resultant from meal ingestion[26,29,55,67]. Thus, the HISS mechanism seems to fit within such gut-brain-liver axis. Recent data obtained by our group further supports the involvement of HISS in this gut-brain-liver axis, since we recently observed that the intestine plays a key role in the process of postprandial activation of HISS pathway, through absorption of substrates required for GSH synthesis and stimulation of PNS (Afonso and Macedo, unpublished data). These data also seem to fit in the hypothesis previously launched by Lautt, according to whom HISS pathway results from a centrally-mediated reflex[45]. In this context, we propose the involvement of the gut-brain axis in hepatic activation of HISS synthesis/release.

Gut-brain axis depends on vagal stimulation, but it is described only in control of exocrine pancreas secretions and enteric motility, secretion and flux[77,78]. Additionally, other authors observed that this gut-brain reflex also affects the liver, although such description is restricted to hepatic glucose production[80]. Therefore, considering these and our results, one can speculate about the existence of an association between the gut-brain axis and the hepatic mechanism of insulin sensitization.

In fact, insulin action increases after ingestion of a mixed-meal, through a mechanism that depends on hepatic parasympathetic nerves, in resemblance with gut-brain reflex, also vagally mediated[77,78]. Furthermore, postprandial potentiation of insulin action seems to be triggered in the upper intestine (duodenum), which suggests the existence of a prandial reflex initiated in the gut following a mixed-meal. Such prandial reflex may be centrally mediated and involves the parasympathetic nervous system, which, along with high levels of hepatic GSH, activates the hepatic mechanism of HISS synthesis and/or release, promoting peripheral insulin action. This hypothesis is represented in figure 2.

FIGURE 2



Proposed hypothesis for activation of the hepatic insulin sensitizing substance (HISS) pathway following a meal. Intestinal nutrient absorption after a mixed-meal (composed of proteins, lipids and carbohydrates) allows glutathione (GSH) synthesis that is essential for synthesis/release of HISS; simultaneously, a postprandial mechanism is triggered in the intestine, which seems to involve central nervous system (CNS) and stimulates the liver, through parasympathetic nerves, for HISS synthesis and release – entero-cerebral-hepatic hypothesis. HISS then acts at the skeletal muscle to potentiate insulin-dependent glucose uptake. Cys, cysteine; ⊕ activation. Adapted from[81].

CONCLUSION

Insulin plays a central role in carbohydrate metabolism. Normal insulin action is physiologically required to regulate glucose homeostasis. Pathological insulin resistance constitutes the most important and serious event in the progression towards diabetes in

such a way that most therapeutic strategies are firstly aimed at increasing peripheral insulin sensitivity. So far, most of these strategies have been supported by insulin sensitivity studies based on a steady-state approach, *i.e.*, fasted state. However, huge differences in terms of insulin sensitivity/resistance and glucose homeostasis have been recently acknowledged between fasting and postprandial conditions. In fact, based on observations that alterations in carbohydrates metabolism occur firstly and more dramatically in the postprandial state, IDF as recently issued guidelines that aim specifically at the management of postprandial glycemia.

Insulin action depends not only on insulin action *per se*, *i.e.*, direct insulin action in its receptor, but also on an hepatic mechanism (HISS) that is responsible for potentiation of peripheral (extra-hepatic)insulin action following a meal. This mechanism, which involves parasympathetic activation and seems to be triggered at the intestine, is active only in the postprandial state. Furthermore, this meal-induced insulin sensitization seems to be sensitive to meal composition. Mixed-meals, composed of proteins, lipids and carbohydrates, are capable of triggering the meal-induced insulin sensitization, unlike carbohydrates alone (*e.g.*, glucose or sucrose), which do not trigger this mechanism. Thus, insulin sensitivity increases significantly following a mixed-meal, but not after glucose. Therefore, testing insulin sensitivity either in the fasted state or after a glucose meal neglects the contribution of the hepatic (HISS)-dependent mechanism for insulin action, possibly leading to false negatives in detection of insulin resistance.

Hopefully, the observations reviewed herein will re-direct our clinical approach towards postprandial evaluation of insulin resistance, rendering better results both in terms of early diagnosis and treatment of insulin resistance.

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